

Systematic review



# Speckle tracking analysis: a new tool for left atrial function analysis in systemic hypertension: an overview

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**Speckle tracking echocardiography (STE) is an imaging technique applied to the analysis of left atrial function. STE provides a non-Doppler, angle-independent and objective quantification of left atrial myocardial deformation. Data regarding feasibility, accuracy and clinical applications of left atrial strain are rapidly gathering. This review describes the fundamental concepts of left atrial STE, illustrates its pathophysiological background and discusses its emerging role in systemic arterial hypertension.**

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## Left atrium pathophysiology

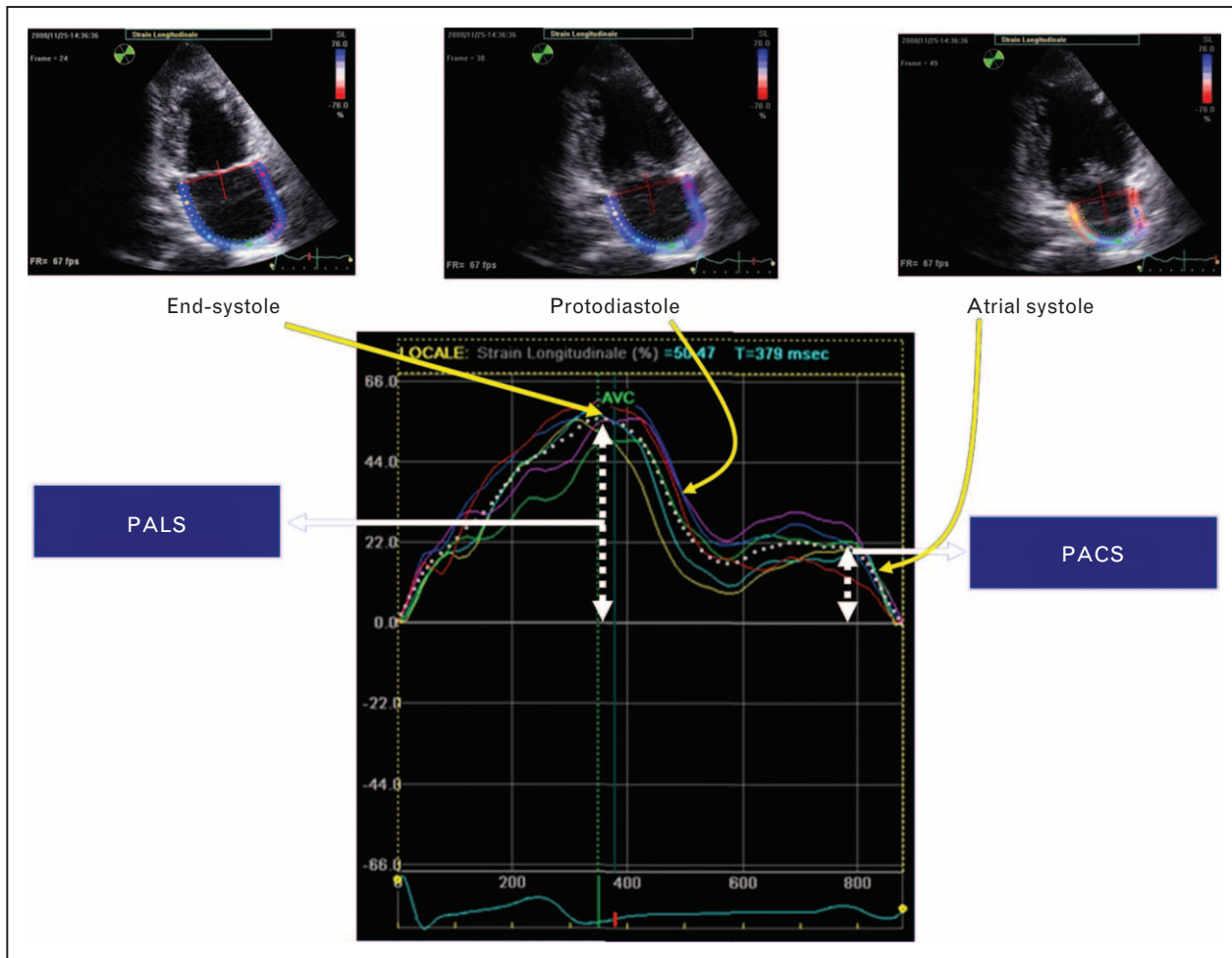
The left atrium, far from a passive transport chamber as frequently assumed, modulates left ventricular filling by acting as a reservoir during left ventricular systole, by allowing blood transfer from pulmonary veins to the left ventricle during early diastole, by augmenting left ventricle filling through its contraction in late diastole and exerting a self-refilling suction source. In addition to its mechanical properties, left atrium contributes to body fluid volume regulation by releasing natriuretic peptides in response to atrial stretch, thus inducing natriuresis, vasodilatation and inhibition of the sympathetic nervous system and renin-angiotensin-aldosterone system inhibition.<sup>1,2</sup>

Through its involvement in total left ventricle stroke volume generation, left atrium function maintains cardiac output and systemic perfusion when the left ventricle is dysfunctional and the loss of its mechanical contribution to left ventricle filling during atrial fibrillation may lead to symptomatic deterioration.<sup>3</sup> In turn, left atrium function is sensitive to changes in left ventricle compliance in both normal<sup>4</sup> and hypertensive states.<sup>5</sup> Moreover, a body of accumulating evidence suggests a role for left atrium function indices as prognostic predictors, a knowledge consolidated by recent advances in the characterization and quantification of left atrium function by non-invasive imaging.

## Echocardiographic evaluation of the left atrium

At present, echocardiography represents the simplest, least invasive and most cost-effective method for the study of left atrium phasic function. Echocardiographic tools for the evaluation of left atrium function include two and three-dimensional echocardiography, Doppler analysis of transmitral and pulmonary vein flow, Tissue Doppler assessment of left atrium myocardial velocities and Speckle tracking echocardiography (STE), as reviewed in recent publications, which the interested reader is referred to.<sup>6</sup> In particular, STE, on which this overview is focused, uses tracking of standard B-mode-generated, angle-independent acoustic speckles for real-time quantitative assessment of regional myocardial deformation. Although originally applied to the analysis of left ventricle function,<sup>7,8</sup> STE-derived measures of atrial longitudinal strain during the cardiac cycle provide the first feasible and reproducible comprehensive functional assessment of atrial walls.<sup>9–13</sup> Thus, STE generates longitudinal strain curves for each atrial segment and a mean dashed curve reflecting the overall atrial function, as depicted in Fig. 1. During the reservoir phase, strain increases as a consequence of stretching in response to left atrium filling, reaching a positive peak just before mitral valve opening, the so-called peak atrial longitudinal strain (PALS). By emptying left atrium, mitral valve opening causes a decrease in strain followed by a second

Fig. 1



Measurement of peak atrial longitudinal strain (PALS) from an apical four-chamber view. The dashed curve represents the average atrial longitudinal strain along the cardiac cycle.

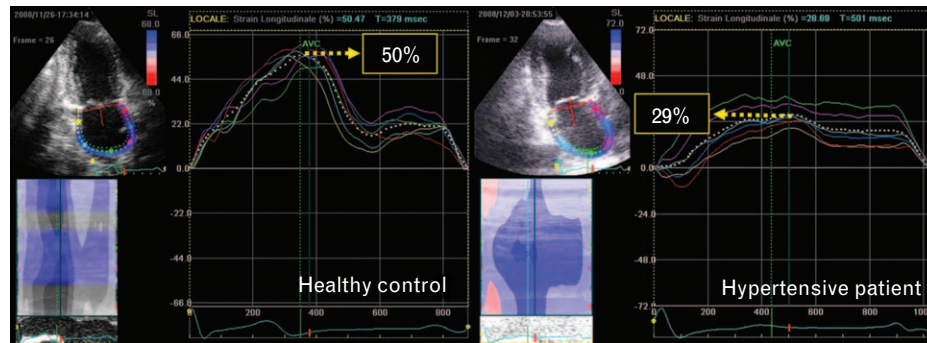
less-positive peak corresponding to the period preceding the atrial contraction, the so-called peak atrial contraction strain (PACS), and finally a negative peak after the atrial contraction.<sup>6</sup> PALS and PACS represent the two main quantitative indices of STE-derived left atrium function (Fig. 1).

#### Left atrial strain in hypertension

A number of studies have been performed using this novel imaging technique for the assessment of atrial function in hypertension. A negative impact of hypertension and diabetes on left atrium function was previously demonstrated by assessment of phasic left atrium volumes and by Doppler echocardiographic measures.<sup>14,15</sup> Mondillo *et al.*<sup>16</sup> expanded those findings by showing that impaired left atrium strain in patients with hypertension can be detected by STE even in the presence of normal left atrium size suggesting that dysfunction precedes dilatation as assessed by traditional

two-dimensional measures (Figs. 2 and 3).<sup>16</sup> That adverse pattern, more evident in non-dipper hypertensive patients,<sup>17</sup> is perhaps a reflection of increased left ventricle pressure secondary to the systemic hemodynamic overload,<sup>18</sup> although other factors may be involved. In fact, the altered atrial strain was reported in hypertensive patients even in absence of ventricular remodeling and early signs of diastolic dysfunction (Fig. 4).<sup>19</sup> Moreover, atrial function was preserved in elite athletes with physiological left ventricle hypertrophy (H), quite in contrast with hypertensive patients despite an equivalent degree of left ventricle remodeling. Athletes presented a peculiar left atrium strain pattern characterized by a shift in the ventricular filling period toward early diastole; this shift leads to a more rapid passive atrial emptying, which is associated with a lower global PACS in athletes compared with controls. This phenomenon may likely be related to an increased flexibility and elasticity of the left ventricle muscle<sup>20</sup> and increased myocardial compliance

Fig. 2



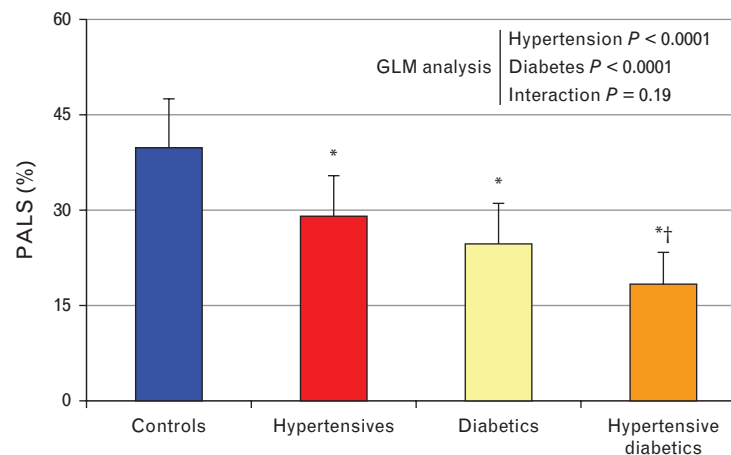
Peak atrial longitudinal strain (PALS) and peak atrial contraction strain (PACS) in a representative control subject as compared with a hypertensive patient.

at the end of diastole in athletes and it suggests that STE analysis may help to clarify the role of left atrium function in the context of physiological left ventricular hypertension (LVH).<sup>21,22</sup> Similarly, two-dimensional atrial strain was significantly reduced in patients with hypertrophic cardiomyopathy (HCM) as compared with non-HCM left ventricle hypertrophy and healthy volunteers,<sup>23</sup> confirming the additive value of two-dimensional atrial strain in the clinical and pathophysiological characterization of LVH.

The study of left atrium function may also guide the choice of anti-hypertensive treatment and its optimization even in the absence of left ventricle dysfunction and/or left atrium dilatation<sup>24</sup> at least according to the results showing a beneficial effect of renin-angiotensin system inhibitors on left atrium strain as opposed to the unchanged standard left atrium echocardiographic parameters.<sup>25,26</sup>

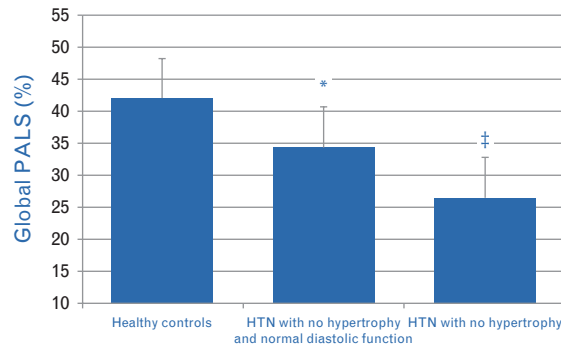
Atrial fibrillation is a frequent event in hypertensive patients<sup>27</sup> in whom an abnormal STE-derived left atrium longitudinal strain and strain rate associate with incident paroxysmal atrial fibrillation<sup>28</sup> independent of left atrium enlargement.<sup>29</sup> Preserved atrial strain and strain rate also predict sinus rhythm maintenance after successful cardioversion<sup>30</sup> as opposed to the increased post-ablation atrial fibrillation recurrence rate in patients with an abnormal left atrium strain.<sup>31</sup> In patients with heart failure, another condition strongly associated with hypertension history,<sup>32</sup> left atrium strain allows to distinguish heart failure with preserved ejection fraction from compensated hypertension with comparable LVH and left atrium dilatation.<sup>33</sup> Left atrium longitudinal strain also correlates with pulmonary capillary wedge pressure in advanced chronic heart failure<sup>34,35</sup> in which an increased left ventricle filling pressures drive left atrium remodelling. Quite notably, left atrium systolic deformation was more depressed in idiopathic than ischemic cardiomyopathy,

Fig. 3



Peak atrial longitudinal strain (PALS) in healthy controls, hypertensive patients, patients with diabetes and hypertensive patients with diabetes (Readapted from<sup>16</sup>).

Fig. 4



Peak atrial longitudinal strain (PALS) in healthy controls, hypertensives without hypertrophy or diastolic dysfunction and hypertensives without hypertrophy with diastolic dysfunction (Readapted from<sup>19</sup>).

perhaps as a result of an extension of the pathological process to left atrium.<sup>36,37</sup>

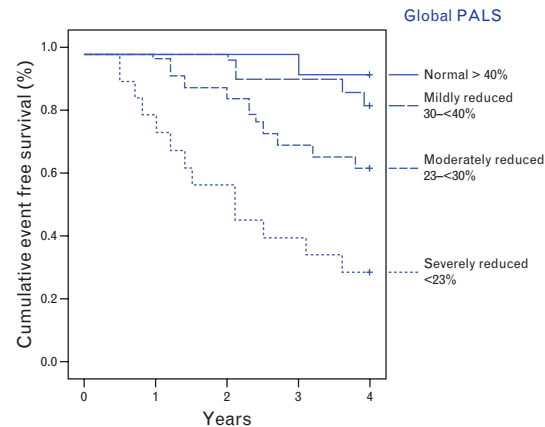
#### Determinants of left atrium strain

The extent of active, passive and conduit filling by the atrium is significantly influenced by the compliance of the left ventricle. In the normal aging process, there is a decrease in passive left atrium emptying and conduit volumes together with an increase in active atrial emptying; these changes are probably because of compensatory mechanism to overcome the normal age-related decrease in left ventricle relaxation.<sup>38</sup> In this view, the assessment of left atrium longitudinal strain appears as a composite measure of left ventricle diastolic function. In fact, reduced PALS was associated with measures of left ventricle function in acute myocardial infarction<sup>39</sup>; however, left atrium strain shows its exclusive in case of increased preload, and in advanced systolic heart failure, it predicted left ventricle preload more effectively than more conventional measurements of diastolic function.<sup>35</sup> Structural atrial remodeling represents an additional morpho-functional correlate of left atrium strain and a close correlation exists between PALS and left atrium myocardial fibrosis in contrast with the loose association with left atrium volume.<sup>40</sup> The conclusion is consonant with data obtained by delayed-enhancement MRI in patients with either paroxysmal or permanent atrial fibrillation.<sup>41</sup>

#### left atrium and prognosis

Large population-based studies have demonstrated the long-term prognostic value of conventional left atrium quantification in cardiovascular disease<sup>42–47</sup> and more recent studies have extended that evidence to left atrial deformation analysis<sup>48,49</sup> highlighting the potential of this technique as an independent prognosticator more effective than conventional left atrium indices (Fig. 5).<sup>47</sup> In addition, patients with mitral regurgitation with reduced left atrium strain presented higher incidence of paroxysmal atrial fibrillation episodes;<sup>50</sup> patients with chronic

Fig. 5



Graded relationship between Kaplan–Meier cumulative event-free survival and categorical increment of global peak atrial longitudinal strain (global PALS) (Readapted from<sup>47</sup>).

atrial fibrillation and lower levels of left atrium strain have higher risk of cardio-embolic events.<sup>51–53</sup>

#### Limitations

The measurement of global PALS assessment, although feasible in most cases, relies quite heavily upon adequate apical views and operator skills and is evidently less accurate in patients with non-sinus rhythm, requiring the average value of almost five consecutive beats.<sup>9</sup>

#### Conclusion

Assessment of left atrium function by STE provides more detailed information about left atrium mechanics and may have an important clinical impact in hypertension and related conditions, thus enabling the development of more effective strategies for cardiovascular treatment and prevention.

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#### References

- Blume GG, Mcleod CJ, Barnes ME, *et al.* Left atrial function: physiology, assessment, and clinical implications. *Eur J Echocardiogr* 2011; **12**:421–430.
- Roelandt J. The increasing importance of left atrial size assessment. *J Cardiovasc Med* 2011; **12**:14.
- Leung DY, Boyd A, Ng AA, *et al.* Echocardiographic evaluation of left atrial size and function: current understanding, pathophysiologic correlates, and prognostic implications. *Am Heart J* 2008; **156**:1056–1064.
- Thomas L, Levett K, Boyd A, *et al.* Compensatory changes in atrial volumes with normal aging: is atrial enlargement inevitable? *J Am Coll Cardiol* 2002; **40**:1630–1635.
- Mattioli AV, Bonatti S, Monopoli D, *et al.* Influence of regression of left ventricular hypertrophy on left atrial size and function in patients with moderate hypertension. *Blood Press* 2005; **14**:273–278.
- Cameli M, Lisi M, Righini FM, Mondillo S. Novel echocardiographic techniques to assess left atrial size, anatomy and function. *Cardiovasc Ultrasound* 2012; **10**:4. DOI: 10.1186/1476-7120-10-4.



- 7 Mondillo S, Galderisi M, Mele D, et al., Echocardiography Study Group Of The Italian Society Of Cardiology (Rome, Italy). Speckle-tracking echocardiography: a new technique for assessing myocardial function. *J Ultrasound Med* 2011; **30**:71–83.
- 8 Cameli M, Ballo P, Righini FM, et al. Physiologic determinants of left ventricular systolic torsion assessed by speckle tracking echocardiography in healthy subjects. *Echocardiography* 2011; **28**:641–648.
- 9 Cameli M, Caputo M, Mondillo S, et al. Feasibility and reference values of left atrial longitudinal strain imaging by two-dimensional speckle tracking. *Cardiovasc Ultrasound* 2009; **7**:6.
- 10 Sirbu C, Herbots L, D'hooge J, et al. Feasibility of strain and strain rate imaging for the assessment of regional left atrial deformation: a study in normal subjects. *Eur J Echocardiogr* 2006; **7**:199–208.
- 11 Okamatsu K, Takeuchi M, Nakai H, et al. Effects of aging on left atrial function assessed by two-dimensional speckle tracking echocardiography. *J Am Soc Echocardiogr* 2009; **22**:70–75.
- 12 Padeletti M, Cameli M, Lisi M, et al. Reference values of right atrial longitudinal strain imaging by two-dimensional speckle tracking. *Echocardiography* 2012; **29**:147–152.
- 13 Di Salvo G, Pacileo G, Castaldi B, et al. Two dimensional strain and atrial function: a study on patients after percutaneous closure of atrial septal defect. *Eur J Echocardiogr* 2009; **10**:256–259.
- 14 Eshoo S, Boyd AC, Ross DL, et al. Strain rate evaluation of phasic atrial function in hypertension. *Heart* 2009; **95**:1184–1191.
- 15 Ballo P, Cameli M, Mondillo S, et al. Impact of diabetes and hypertension on left ventricular longitudinal systolic function. *Diabetes Res Clin Pract* 2010; **90**:209–215.
- 16 Mondillo S, Cameli M, Caputo ML, et al. Early detection of left atrial strain abnormalities by speckle-tracking in hypertensive and diabetic patients with normal left atrial size. *J Am Soc Echocardiogr* 2011; **24**:898–908.
- 17 Açar G, Bulut M, Arslan K, et al. Comparison of left atrial mechanical function in nondipper versus dipper hypertensive patients: a speckle tracking study. *Echocardiography* 2013; **30**:164–170.
- 18 Miyoshi H, Oishi Y, Mizuguchi Y, et al. Effect of an increase in left ventricular pressure overload on left atrial-left ventricular coupling in patients with hypertension: a two-dimensional speckle tracking echocardiographic study. *Echocardiography* 2013; **30**:658–666.
- 19 Cameli M, Lisi M, Righini FM, et al. Early effects of arterial hypertension: left atrial deformation analysis by twodimensional speckle tracking echocardiography. *Int Cardiovasc For J* 2013.
- 20 Santoro A, Caputo M, Antonelli G, et al. Left ventricular twisting as determinant of diastolic function: a speckle tracking study in patients with cardiac hypertrophy. *Echocardiography* 2011; **28**:892–898.
- 21 D'Ascenzi F, Cameli M, Zacà V, et al. Supernormal diastolic function and role of left atrial myocardial deformation analysis by 2D speckle tracking echocardiography in elite soccer players. *Echocardiography* 2011; **28**:320–326.
- 22 Claessens PJ, Claessens CW, Claessens MM, et al. Supernormal left ventricular diastolic function in triathletes. *Text Heart Inst J* 2001; **28**:102–110.
- 23 Paraskevaidis IA, Panou F, Papadopoulos C, et al. Evaluation of left atrial longitudinal function in patients with hypertrophic cardiomyopathy: a tissue Doppler imaging and two-dimensional strain study. *Heart* 2009; **95**:483–489.
- 24 Kokubu N, Yuda S, Tsuchihashi K, et al. Noninvasive assessment of left atrial function by strain rate imaging in patients with hypertension: a possible beneficial effect of renin-angiotensin system inhibition on left atrial function. *Hypertens Res* 2007; **30**:13–21.
- 25 Dimitroula H, Damvopoulou E, Giannakoulas G, et al. Effects of renin-angiotensin system inhibition on left atrial function of hypertensive patients: an echocardiographic tissue deformation imaging study. *Am J Hypertens* 2010; **23**:556–561.
- 26 Kucukler N, Kurt IH, Topaloglu C, et al. The effect of valsartan on left ventricular myocardial functions in hypertensive patients with left ventricular hypertrophy. *J Cardiovasc Med (Hagerstown)* 2012; **13**:181–186.
- 27 Stewart S, Hart CL, Hole DJ, McMurray JJ. Population prevalence, incidence, and predictors of atrial fibrillation in the Renfrew/Paisley study. *Heart* 2001; **86**:516–521.
- 28 Tsai WC, Lee CH, Lin CC, et al. Association of left atrial strain and strain rate assessed by speckle tracking echocardiography with paroxysmal atrial fibrillation. *Echocardiography* 2009; **26**:1188–1194.
- 29 Kojima T, Kawasaki M, Tanaka R, et al. Left atrial global and regional function in patients with paroxysmal atrial fibrillation has already been impaired before enlargement of left atrium: velocity vector imaging echocardiography study. *Eur Heart J Cardiovasc Imaging* 2012; **13**:227–234.
- 30 Rondano E, Dell'Era G, De Luca G, et al. Left atrial asynchrony is a major predictor of 1-year recurrence of atrial fibrillation after electrical cardioversion. *J Cardiovasc Med* 2010; **11**:499–506.
- 31 Hong J, Gu X, An P, et al. Left atrial functional remodeling in lone atrial fibrillation: a two-dimensional speckle tracking echocardiography study. *Echocardiography* 2013; **30**:1051–1060.
- 32 Levy D, Larson MG, Vasan RS, et al. The progression from hypertension to congestive heart failure. *JAMA* 1996; **275**:1557–1562.
- 33 Kurt M, Wang J, Torre-Amione G, Nagueh SF. Left atrial function in diastolic heart failure. *Circ Cardiovasc Imaging* 2009; **2**:10–15.
- 34 Wakami K, Ohte N, Asada K, et al. Correlation between left ventricular end-diastolic pressure and peak left atrial wall strain during left ventricular systole. *J Am Soc Echocardiogr* 2009; **22**:847–851.
- 35 Cameli M, Lisi M, Mondillo S, et al. Left atrial longitudinal strain by speckle tracking echocardiography correlates well with left ventricular filling pressures in patients with heart failure. *Cardiovasc Ultrasound* 2010; **8**:14.
- 36 D'Andrea A, Caso P, Romano S, et al. Association between left atrial myocardial function and exercise capacity in patients with either idiopathic or ischemic dilated cardiomyopathy: a two-dimensional speckle strain study. *Int J Cardiol* 2009; **132**:354–363.
- 37 D'Andrea A, Caso P, Romano S, et al. Different effects of cardiac resynchronization therapy on left atrial function in patients with either idiopathic or ischaemic dilated cardiomyopathy: a two-dimensional speckle strain study. *Eur Heart J* 2007; **28**:2738–2748.
- 38 Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part II: the aging heart in health: links to heart disease. *Circulation* 2003; **107**:346–354.
- 39 Ersbøll M, Andersen MJ, Valeur N, et al. The prognostic value of left atrial peak reservoir strain in acute myocardial infarction is dependent on left ventricular longitudinal function and left atrial size. *Circ Cardiovasc Imaging* 2013; **6**:26–33.
- 40 Cameli M, Lisi M, Righini FM, et al. Usefulness of atrial deformation analysis to predict left atrial fibrosis and endocardial thickness in patients undergoing mitral valve operations for severe mitral regurgitation secondary to mitral valve prolapse. *Am J Cardiol* 2013; **111**:595–601.
- 41 Kuppahally SS, Akoum N, Burgon NS, et al. Left atrial strain and strain rate in patients with paroxysmal and persistent atrial fibrillation: relationship to left atrial structural remodeling detected by delayed-enhancement MRI. *Circ Cardiovasc Imaging* 2010; **3**:231–239.
- 42 Maiello M, Sharma RK, Ciccone MM, et al. Differential left atrial remodeling in LV diastolic dysfunction and mitral regurgitation. *Echocardiography* 2009; **26**:772–778.
- 43 Tsang TS, Barnes ME, Gersh BJ, et al. Left atrial volume as a morpho-physiologic expression of left ventricular diastolic dysfunction and relation to cardiovascular risk burden. *Am J Cardiol* 2002; **90**:1284–1289.
- 44 Tsang TS, Abhayaratna WP, Barnes ME, et al. Prediction of cardiovascular outcomes with left atrial size: is volume superior to area or diameter? *J Am Coll Cardiol* 2006; **47**:1018–1023.
- 45 Meris A, Amigoni M, Uno H, et al. Left atrial remodeling in patients with myocardial infarction complicated by heart failure, left ventricular dysfunction or both: the VALIANT Echo study. *Eur Heart J* 2009; **30**:56–65.
- 46 Pedrinelli R, Ballo P, Fiorentini C, et al. Gruppo di Studio Ipertensione e Cuore, Società Italiana di Cardiologia. Hypertension and acute myocardial infarction: an overview. *J Cardiovasc Med (Hagerstown)* 2012; **13**:194–202.
- 47 Pedrinelli R, Ballo P, Fiorentini C, et al. Gruppo di Studio Ipertensione e Cuore, Società Italiana di Cardiologia. Hypertension and stable coronary artery disease: an overview. *J Cardiovasc Med (Hagerstown)* 2013; **14**:545–552.
- 48 Antoni ML, Ten Brinke EA, Atary JZ, et al. Left atrial strain is related to adverse events in patients after myocardial infarction treated with primary percutaneous coronary intervention. *Heart* 2011; **97**:1332–1337.
- 49 Cameli M, Lisi M, Focardi M, et al. Left atrial deformation analysis by speckle tracking echocardiography for prediction of cardiovascular outcomes. *Am J Cardiol* 2012; **110**:264–269.
- 50 Cameli M, Lisi M, Righini FM, et al. Left atrial speckle tracking analysis in patients with mitral insufficiency and history of paroxysmal atrial fibrillation. *Int J Cardiovasc Imaging* 2012; **28**:1663–1670.
- 51 Shih JY, Tsai WC, Huang YY, et al. Association of decreased left atrial strain and strain rate with stroke in chronic atrial fibrillation. *J Am Soc Echocardiogr* 2011; **24**:513–519.
- 52 Mohammed I, Mohmand-Borkowski A, Burke JF, Kowey PR. Stroke prevention in atrial fibrillation. *J Cardiovasc Med (Hagerstown)* 2012; **13**:73–85.
- 53 Saha SK, Anderson PL, Caracciolo G, et al. Global left atrial strain correlates with CHADS2 risk score in patients with atrial fibrillation. *J Am Soc Echocardiogr* 2011; **24**:506–612.

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